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VARIATIONS WITHIN A BACTERIAL SPECIES—I MORPHOLOGIC VARIATIONS [1]

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(WITH PLATES 29–33)

It would seem redundant to call attention to the fact that bacteria, including phytopathogens, may be expected to vary. Many articles, including extensive monographs, have been written on the subject; a symposium was devoted to it at the recent International Botanical Congress held at Ithaca, New York, and yet, in spite of this, the current literature on bacterial plant pathogens shows clearly that very little recognition has been given to this phenomenon. It is true that most of the work dealing with bacterial variability has appeared in medical journals and in other periodicals and books which are ordinarily not available to plant pathologists and mycologists; likewise, from a reading of the literature it is quite obvious that medical bacteriologists have paid very little attention to the literature of mycologists and plant workers in general and have not paid sufficient attention to morphological details. The article here presented and one to be given later will attempt in a limited fashion to bridge this gap and will call attention to several cases of variability within a species as it has been observed by the writer in certain bacterial plant parasites, in a human parasite and in several saprophytes.

What is meant by variation in a bacterial species? Is it something, more or less ephemeral, that is associated with or brought about by differences in environment, is it inheritable, or is it simply a phase or a part of a "life cycle"? Such questions are

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not as likely to be asked by a biologist dealing with a variation in a pure line of tomatoes, for example, as they are by a bacteriologist. Why? Because in the first place it is a relatively simple matter to obtain a homozygous individual belonging to certain groups of higher plants or animals, compared with the difficulties of isolating a minute, single-celled individual, and, unless one does isolate a single bacterium and observes its offspring, how can it be certain that a pure line and not a mixed one has been obtained? In the second place, a number of bacteriologists now insist that the so-called fission-fungi have a far more complex life history than has been attributed to them in the past. Hence, it is not at all clear that any deviation may not represent a phase which, while differing from that obtainable under "normal" conditions, nevertheless represents a distinct part of the life history. To illustrate the point, it is very well known that, in various species of fungi, cultural conditions which make for so-called normal growth preclude the possibility of producing such structures as perithecia, zygospores, chlamydospores, sclerotia and other well-defined types of bodies. It is only when nutrients run low, or when the temperature is "abnormal" or when a chemical or physical stimulant is applied that certain structures are obtained which are really just as normal to the species at hand as the so-called normal phases. Would it not be more correct to say that when we isolate a microörganism and give it facilities for making profuse growth of a certain recognized type it is under abnormal conditions? How often under natural conditions will a given microörganism be found to have a substratum all to itself, or to have the same light for any length of time, the same temperature, or the same humidity? Even a pathogen, once it penetrates and occupies a portion of a host, must soon compete with secondary invaders for its supremacy in the occupied area, and may not the secondary invader so check the primary one as to inhibit its further invasion into unoccupied territory? The writer has evidence to show that this happens at times to *Bacillus amylovorus* and that a yellow schizomycete, entirely unrelated to the fire blight pathogen, is with astounding frequency to be found following up the work of the primary parasite and apparently limiting it in the amount of host tissue

invaded. This has been found to occur not only in young, succulent pear and apple twigs, but also in green pear fruit. In passing it may be worth while to note that this is a field of endeavor that has hardly been touched in spite of the fact that it gives promise of yielding extremely interesting scientific as well as practical knowledge. We are so anxious to obtain the parasite and nothing but the parasite in pure culture that we have not taken the trouble to find out something about the natural companions of the pathogen and whether they are desirable or undesirable from the standpoint of the parasite and from the standpoint of the host. In brief, when we speak of normal growth or of normal growing conditions of a bacterium or a fungus we may be designating a more or less abnormal and unnatural state, and, to cite a specific case, when it is found that perithecia of *Venturia inaequalis* are not produced in pure cultures unless the mycelium is violently treated, either severely cut into bits or subjected to the action of a contaminant, we do not assume that these structures are abnormal or atypical. Likewise, it is highly improper to speak of abnormal or involution forms in bacteria because they are produced under conditions which are not considered ideal for the production of a standard type of growth. But, while a great deal has been written about various types of structures in bacteria, such as gonidia, buds, sex organs, zygospores, symplasm and the like, they are still shrouded in mystery in spite of the splendid work of a relatively large number of bacteriologists, including Löhnis (**28**, **29**, **30**), Hadley (**22**), Pringsheim (**39**), Enderlein (**17**), Mellon (**32**, **33**, **34**), Gurney-Dixon (**21**), Hort (**23**, **24**, **25**), Baerthlein (**3**), and Eisenberg (**10** to **16**). In view of this uncertainty bacteriologists cannot easily decide whether a given form, designated a variant, is comparable to those variants in pure line work in higher plants and animals or whether it is merely another stage in the life history of the organism.

Assuming that bacteria have a far more complex life history than has formerly been recognized and that they are pleomorphic or "cyclogenic," is there not still the possibility that, given any one form or phase of the cycle we may expect it to show variation? In other words, granted that one phase or form of a particular species may produce gas and another phase of the

same species show no gas production, the possibility still exists that there may be clones or strains of the organism which in comparable phases may or may not produce gas or which may vary in a number of other particulars, morphologically and physiologically. Hadley (**22**), who has written a splendid monograph on variation in bacteria, prefers not to think of it from this viewpoint. "Not until we have become able to recognize the wide range of cyclogenic variation to which all bacterial species are susceptible, shall we be able to detect the permanent departure from the specific cycle" (p. 273). Then again, "The most important point . . . is that the cyclogeny of a single bacterial species embraces many strictly normal forms of culture growth, each of which is endowed with different biochemical, serologic and antigenic characters" (p. 272).

The possibility that within a recognized species of bacteria there may be a number of races, strains or varieties has been set forth from time to time by various bacteriologists. Some twelve years ago Cole and Wright (**6**) discussed this subject, applying the pure line concepts of geneticists to bacterial species. They present an excellent summary of some of the literature on this subject, paying special attention to the application of the statistical method by Andrews and Horder, Bredemann, Goodman, Winslow, Winslow and Walker, Wolf, and Buchanan and Truax, in the study of bacterial species. Cole and Wright concluded that the studies of the investigators just mentioned "have emphasized what was already known from qualitative studies, that within recognized species there are distinct cultural races or varieties, each with its own characteristics and range of variability, and that these may exist side by side, independent of the environmental conditions." They further cite a statement made by Winslow in 1908, that "bacterial types (species) should never be described on the strength of an examination of a single individual strain, but only after a comparative study of the numerical frequency of each particular character in a considerable series of cultures." Although he prefers to view the variations shown by any particular species of bacteria as primarily associated with its cyclic development, nevertheless Hadley (**22**) has not entirely overlooked the possibilities of racial or varietal development within a

species. He says: "It is perhaps true that, of all the variations shown by different strains, many are due to the isolation of biotypes, each possessing its own range of variation, and probably overlapping other ranges."

MORPHOLOGICAL VARIATIONS

In an address delivered in 1924 before the Mycological Section of the American Botanical Society, at the Cincinnati meeting, entitled "Some Evidence for Gonidia Formation in Certain Bacteria," the writer showed a relatively large number of lantern slides, representing micro-photographs depicting morphological variations within a bacterial species. In the meantime there have appeared a number of articles and books (**2**, **32**, **33**, **34**), some of which show figures quite comparable to those that the writer has found. These authors without exception have interpreted the various forms shown by any one species as indicating complexities in the life cycle which had not been previously recognized in an adequate fashion. Gonidia, buds, sex organs, zygospores, coccus and rod forms, mycelioid and single-celled individuals, have been envisioned in species that have in the past been considered as monomorphic. The work of Mellon is particularly worthy of attention. In a series of papers he has brought to light some interesting data concerning the morphological and cultural variations in a number of bacteria. He isolated a strain of *B. coli* from a patient suffering from pyelitis which "grew with such pleomorphism as to suggest a fungus." It had long filaments, some of which were branched, and many very large coccus-like forms, which developed from the filaments. For staining he used carbol-thionin with which "fixation artifacts are . . . avoided." The interesting thing about this strain was that the pleomorphism quickly disappeared when the patient no longer received urotropin and only normal-looking *B. coli* were to be found. But when the drug was again administered the fungoid filaments immediately reappeared. Grown on Endo agar at 37° C. from a single cell isolation, the plate colonies which developed showed the fungoid form of the organism. Preparations of such colonies showed a peculiar arching or tilting of two adjoining cells of a filament resulting in an apparent union of the arched

portions and giving rise to a globoid structure at the point of union. It is these roundish bodies which he takes to be zygospores. While his micro-photographs do not reveal the details of the union nor of the cytological phenomena which may be involved, nevertheless they present an almost convincing demonstration of a union of two cells. Assuming that this is a sexual act, the term "zygospore" which Mellon applies is to be questioned, although the photographs seemingly show gametes of equal size. But to compare this with the sexual process in yeasts, as Mellon does, is to overlook the subsequent development of a rather highly developed fungus with its asci and ascospores, although, superficially at least, the apparent union itself is very comparable to that which Mellon finds in this bacillus.

The closest approach of these "zygospores" to any fungus structure which the writer has found is that shown by Zopf (**41**) and Dangeard (**7**) in the Ancylistales, a group of parasitic water-molds recognized by mycologists as representing very simple forms of fungi. In the genus *Myzocytium*, Zopf and Dangeard have each presented drawings of forms in which adjoining cells of a relatively simple thallus may function as sex organs which give rise to an oöspore. Likewise in *Lagenidium*, also representing a very low form of fungus body, adjoining cells of a filament may send out side branches which function as sexual bodies, one as an antheridium, the other as an oögonium. The roundish oöspore is the result of the fusion of these two organs. These are merely suggestions of comparable structures which are to be found in the fungi; but so little is known about the morphology and cytology of the bacterial bodies under discussion that it is very difficult to homologize with any degree of certainty. Aside from the proper designation of these bodies, Mellon has revealed some interesting data about them. They take the form of giant cocci and he regards the arthrospores of the older bacteriological literature as comparable to them physiologically. They are usually to be observed only "under conditions of marked aërobiosis such as pellicle formation and on agar slants." In another article (**33**) he presents the subsequent history of these "zygospores." When a transfer is made from a mucoid colony which gives rise to these roundish bodies, the resulting colony on an agar slant shows only

large coccoidal forms in the condensation water. "Since none of the small, normal-sized (rod-shaped) *B. coli* were present it seems quite clear that these mucoid colonies represented a reproduction of the larger coccoid forms whose immediate antecedents were the zygospores. . . . " When these "zygospores" were inoculated into broth under the warm stage they were observed to reproduce as enormous coccoids but when inoculated into agar they reproduced not as coccoids but as filaments or rods, which in turn gave rise to zygospores. In still another article (**34**) he describes similar structures with comparable life histories in a diphtheroid strain of bacteria. Likewise, he now presents additional studies on the morphology of *B. coli*, with micro-photographs showing gonidia or buds. By staining intravitally he observed unipolar and bipolar buds or gonidia being formed on the rods and in some cases these buds gave rise to secondary ones. These resemble somewhat the figures shown by the present writer (**40**) for bud formation in *B. tumefaciens*, and are also comparable to figures which will be here shown and presently discussed.

While using a bacterial flagella stain, involving a method which has not yet been described, certain peculiar particles were noted which appeared quite frequently in young cultures (PLATE 29) of an organism which is closely related to the ordinary potato bacillus, *B. mesentericus;* the organism is a relatively large, peritrichous, spore-forming rod and, whatever may be its exact identity, it belongs to the group of which the hay bacillus, *B. subtilis*, is the type. It was isolated from a plug of a potato tuber, the tuber having been derived from a plant severely diseased with Irish potato mosaic. As the same organism appeared in several tubes from such plugs, while it was absent in similar tubes in which plugs of disease-free tubers were used, there appeared the possibility that it might be associated with mosaic. However, inoculation experiments were all failures and there is no good reason for believing that the organism is not an ordinary soil inhabitant which, on account of its resistant spores, remained viable and adhered to the surface of some tubers, even after an hour's immersion in a 1 to 1,000 mercuric chloride solution. The particles just mentioned were to be found in both young and old cultures and on various nutrient media.

The frequency with which flagella were to be found attached to these particles seemed surprising (PLATES 29, 30), for, as is well known, flagella are readily lost or cast off from normal rods and, if these particles represent dead or dying bacteria, it is rather odd to find the flagella still adhering to the decomposing material. But what appeared most surprising was the relatively large numbers of apparently disintegrating rods and of particles which were undoubtedly remnants of rods (PLATES 29, 30). Some rods may be seen to be stained deeply with the interiors appearing homogeneous and others may be seen as lighter staining structures with markedly differentiated portions. It is the last-named rods which show particles or granules of various types appearing within the contours of the rod and not infrequently a flagellum may be seen arising from or oriented toward such particles (see PLATES 29, 30). Frequently the rods have disintegrated to such an extent that their outlines have disappeared and the form of the fragments may not lead one to suspect their relationship to the usual rod-shaped figure. But a careful study of many preparations, both in living, hanging-drop cultures and in stained material, leaves no doubt of the relationship of these particles to the rods. They are not artifacts and they are not contaminations. I have studied this organism for more than five years and have made repeated transfers from single colonies and the preparations are consistent throughout.

As to the particles representing remnants or dissociation products of rods there are a number of interesting features associated with them. It has already been noted that flagella are frequently to be found attached to such particles, even when they are free from the mother rod (PLATES 31, 32). These particles or granules vary greatly in size and in shape, some being so tiny that were it not for the subtending flagellum they would be either overlooked or would not be resolved with the highest power of the microscope. The writer has attempted to measure some of the smallest and finds that it is hardly possible to do so even with a Zeiss filar micrometer. They measure less than 0.1 μ and, taking into consideration the increase in size that one expects in mordanting and in depositing a stain, the actual size would be closer to 0.05 μ than to 0.1 μ, or 50.0 $\mu\mu$. It is interesting to compare such sizes

with those given by Duggar and Karrer (**8**) for particles constituting the "contagium vivum fluidum" of the mosaic disease of tobacco. They find that these disease-producing particles are approximately the size of colloidal hemoglobin, which is stated to be between 30 and 40 $\mu\mu$. Thus, aside from any consideration as to the biological significance of the particles under discussion there can be no doubt that they approach the sizes of a filterable virus and yet are observable. Grading upward in size from these minute particles, there are to be found many others which are larger, often to be observed clumped more or less together, some being good-sized portions of a fully developed rod. In shape they vary from a more or less regular roundish, oval, oblong, or cylindrical figure with terete walls to a somewhat irregular structure with no well-defined outline. They stain very readily and heavily in contrast to the walls of the mother rods which stain but lightly in the same staining period, even with carbol-fuchsin. Similar particles have been observed within the rods and free from them in a number of species, though the number of the particles has not been nearly as great as in the species previously mentioned. Among those showing such granules are the following authentic species, *B. typhi* (*B. typhosus*), *B. vulgatus*, *B. mesentericus*, *B. subtilis*, and *Proteus vulgaris* (PLATES 30, 33).

The question which presents itself for consideration with reference to these particles is, are they living entities or merely dead, disintegration products? Those who are acquainted with Löhnis' own work (**29**, **30**) and with the splendid survey of the literature in this field which he has presented (**28**) will recognize in the microphotographs here presented a striking resemblance to many of the figures that he has brought together. In a relatively large number of bacteria he has found certain phases which seemingly indicate a far more complex life history than has been assumed to exist. In this paper we are primarily concerned with those phases which Löhnis interprets as buds, granules or gonidia which represent distinct parts of the life cycle and which he believes are living forms going through a more or less specified developmental process and capable of giving rise to the normally accepted rod or coccus inherent to the respective species. Indeed, it may not be too much to say that from one point of view the figures here shown

add materially to his interpretations and that in very few, if in any, figures which he presents does one see such good pictorial evidence of a bud or gonidial forming process. But are these granules, with their flagella, really alive and how can this be proved?

Aside from the morphological evidence observed in stained preparations there are at least three possible ways of determining the vitality of these granules. First and perhaps most important would be to attempt direct microscopic observations of living preparations; second, to attempt to separate the particles from the large rods by using a bacteriological filter and noting any colony growth which may develop from the filtrate; and third, to attempt to isolate single particles and to determine their ability to grow or to reproduce. All three of these methods have been tried by the writer, the last one only to a limited extent because of the great difficulties involved and the first two in a rather extensive series of microscopic observations and filtrations. The results, which are now to be presented, are not convincing and the writer must say immediately that the evidence now at hand for the belief that the particles are alive is not as good as the writer considered it to be four years ago, although the evidence at that time was not taken to be conclusive.

As to the work dealing with direct microscopic observations, use was made of the finest series of apochromatic lenses available, with various sources of light and with different magnifications, including the highest to be obtained. As the organism grows very rapidly at 25 to 30° C., most of the microscopic work was done in a small transfer chamber where the temperature fluctuated but slightly and approximated or reached the optimum for colony development. Transfers from both young and old cultures were made to sterile cover glasses, the medium being either a three mm. loopful of nutrient broth or of the water of synaeresis from the base of a nutrient agar slant. These were then used as hanging-drop slides in Van Tieghem cells. The rods being relatively large, 0.75 to 1.0 μ by 2.5 to 7.0 μ, were readily observable under the microscope even with 16 mm. objectives and low-powered oculars. The specific objects sought for were fragmented rods or particles which showed true motility or direct ocular evidence

of increase in size or of reproduction of any observable particle, whether it be large or small. That the medium and other environmental factors were suitable for growth in these cells was ascertained by noting the relative number of individuals within the drop after a given length of time. In addition to noting the free particles considerable time was spent in observing the fragmenting rods which still contained one or more granules and in which the original contour of the rod was still to be seen. Briefly, it may be stated that, as a result of making hundreds of such observations, many of which involved continuous staring down the barrel of the microscope for several hours at a stretch with but brief periods of intermission, in not a single case was there clear-cut evidence of increase in size in any observed particle nor was there any evidence of multiplication other than by the ordinary division of the unfragmented rods. As the stained preparations showed these particles to be frequently possessed of flagella, indicating motility, if they were alive, then in living mounts they should move about. While no difficulty whatever was experienced in observing some of the particles and watching them closely for signs of motion, other than showing the usual Brownian movement, only two particles in different cases were seen to be definitely in motion, both of them being within the confines of the old mother-rod wall. When first observed the writer felt that the evidence was complete; one could see the particle in each instance rapidly darting about in a space of about 1.5 by 5.0 μ, impinging first against one end of the old rod wall and then against the other. But, somewhat later while examining a stained preparation mounted in water, one in which osmic acid and carbol-fuchsin were used in the different steps of mordanting and staining, the same sort of motility was observed with even clearer definition. The old rod-wall was lightly stained and the very deeply stained particle could be seen darting back and forth. It is very difficult to believe that this particle was alive and it seems preferable to consider the motion as being the result of some physical process, either in the form of diffusion or osmotic currents of unequal intensity in different parts of the rod, or of irregular temperature expansions and contractions, or of some electrical phenomenon. But, whatever the explanation

may be for this movement, it seems more reasonable to assume that the particle was not alive in this stained preparation and consequently one is forced to conclude that the vitality of the moving particles of the living preparations is open to question. As far as the evidence from direct microscopic observations of living material is concerned it must be conceded that it falls short, to say the least, of proving that the particles were alive.

The next line of evidence to be considered consists of a relatively large number of ultra-filtration experiments. Five different types of bacteriological filters were used, three of which did not permit the passage of dextrin in a one per cent solution, as indicated by the iodine test, and the other two withheld a fresh, one per cent, defibrinated, hemoglobin solution and permitted the passage of only a part of the dextrin. Twenty-four attempts were made to filter young and old nutrient broth cultures, precautions being taken in each case to guard against contaminations. Twelve of these yielded pure cultures of the organism filtered, after a 24 to 48 hours incubation period, nine filtrates remained sterile, and three were thrown out of consideration because transfers from the filtrates did not yield uniform pure cultures and hence were open to the objection that they may represent contaminations. Only the two filters which permitted the passage of a part of the dextrin enabled the organism to pass through the pores. The evidence from these filtration experiments for the passage of some of the particles and their ability to reproduce typical colonies certainly appears substantial. The fact that each of the filters was carefully standardized by means of colloidal hemoglobin and dextrin solutions and that those filters which permitted the passage of the organism possessed pores which partly withhold even part of the dextrin solution, would seemingly indicate that the unfragmented rods could by no conceivable means have passed through the filters. The rods, as previously noted, are exceptionally large, many of them being fully 1.0 μ in the smallest diameter and none are less than around 0.75 μ. If such rods were able to pass through a bacteriological filter under ordinary methods of filtration, using a simple vacuum pump operated from a hydrant, for relatively short periods of time, what would be the chance of obtaining sterile filtrates of any microörganism?

B. prodigiosus, as Mudd (**35**) points out, is almost a classical example of an organism that does not pass through ordinary bacteriological filters and yet this microbe, as given in Bergey's manual (**4**), measures only 0.5 by 0.5 to 1.0 μ. Furthermore, as a check on the method of filtration, sterile nutrient broth at different times was passed through the filters and the transfers from the filtrates were invariably sterile. Likewise, the use of hemoglobin and dextrin solutions for standardization purposes should constitute a far more rigid check on the fineness of a filter than the use of any known microörganism, including the smallest. According to Duggar and Karrer (**8**) and Duggar and Armstrong (**9**) who used these substances in standardizing filters and thus determined the sizes of the particles of the virus causing the mosaic disease of tobacco, colloidal hemoglobin particles measure approximately 30 $\mu\mu$, and dextrin particles measure even less than that. When filters will withhold the passage of colloidal hemoglobin measuring 30 $\mu\mu$, it is almost inconceivable that the same filters would permit an organism to pass through which is at least 25 times as large in the smallest diameter. But, in view of recent work (**35, 36**) indicating that a microbe may or may not pass through a filter, depending upon its motility, upon the electric charge, and upon other factors unrelated to the size of the bacterium, what bacteriologist would be willing to stake his reputation on such filtration experiments as offering conclusive proof for the passage of viable particles and not of unfragmented rods?

The third line of evidence to be considered for the viability of the particles involves the isolation of single particles and of fragmented rods and of observing their ability to produce growth. The difficulties inherent to such a procedure may readily be imagined. It may be of interest to record the manner in which this was attempted. The work was conducted in a completely enclosed transfer chamber which had been heavily steamed and the microscope to be used was thoroughly cleaned with a cloth moistened with a disinfectant. A young culture was so diluted with sterile water that a one-half mm. loopful examined in a hanging drop showed by direct microscopic observation only one or two whole, unfragmented rods, or fragments of

rods. The hanging drops were made on sterilized cover slips which were placed over a sterilized, grooved slide. A direct count of bacteria was thus made of each drop and the slide, handled with sterile forceps, was then placed, drop downwards, on a plate containing nutrient agar or nutrient broth. The greatest difficulty in this method resulted from the necessity of adding sterile water to the edge of the cover slip in order to prevent drying out of the small, hanging drop. The method works very well indeed for picking up rather small fungus spores where lactic acid may be added to the edge of a cover slip to prevent drying and this acid prevents bacterial contaminations. But in the case of this bacterium the lactic acid prevented growth, and when the acid was not used contaminations were discouragingly frequent. Nevertheless a few fragmented rods were successfully isolated and in every case the transferred particles failed to produce a colony. Single, unfragmented rods, on the other hand, produced normal colonies. There is of course the possibility that while some particles may be dead others are alive and that live ones were not present among those which happened to have been transplanted. Although a great deal of time was spent in this sort of work, the evidence appears to the writer to be uncertain, indicating, if anything, that the fragments are not alive. It thus appears that of the three lines of investigation undertaken to determine the biological significance of the fragments, only the filtration experiments lend any appreciable support to the idea that the particles are living and capable of producing growth.

If these particles are not alive, then what does this phenomenon represent? Particles or granules have been observed by various investigators in organisms closely related to the one under discussion. In *B. mesentericus*, *B. subtilis*, and *B. mycoides*, Amato (1) has observed and figured certain granules as occurring within the rods. These he believes to be nuclei which go through a certain cycle. From germinating spores there originate, according to his conception, rods with a single, limited, central body. This divides into two and is followed later on by a regular cell division, giving rise to two daughter cells. Following this the central body in each daughter cell disintegrates into very fine granules followed again by cross-wall formation. The granules

arrange themselves along the periphery of the rod and then coalesce into a heavy-staining, thick, spherical body. This body, with the evacuation of some chromatic substance, which passes toward the poles, eventually becomes the spore. As Amato makes no mention of disintegrating rods and free particles prior to spore formation, there is no tangible basis for comparison with the granules under discussion, although as far as granules within undisintegrated rods are concerned, there are certain points of similarity, notably a peripheral arrangement of particles, which are occasionally to be observed (PLATE 31). Fuhrman (**18**) figures a granulation process as occurring simultaneously with a loss of flagella in *B. subtilis* prior to spore formation. But he, also, fails to show any disintegration of the rods prior to spore formation, nor does he indicate a disintegration which is not connected with spore formation. Quite recently Andervont and Simon (**2**) in studying certain pits that developed in colonies of *B. cereus*, growing upon agar slants, found that the pits were the result of a disintegration process of the non-spore-forming rods. Many of these presented a variable number of rounded projections along the lateral surfaces while "others appeared as mere shells containing granules of variable size and number." They also observed various sized granules free from rods and decided that they represented the contents of rods that had disintegrated. Pit formation was then concluded to be essentially brought about by the disintegration and liberation of the cell contents. They present figures which, while small in size and few in number, show striking resemblances to those here presented and while no pits or "pellucid" areas were observed in the cultures of the organism here studied, nevertheless the process of disintegration appears quite similar, and the organisms, being large, peritrichous spore-formers, are certainly rather closely related. It is also interesting to note that out of four trials they were able to obtain two filtrates through Berkefeld V and Berkefeld N candles which after a week's incubation gave rise to growths that were identical with the original culture and they suggest that the disintegration process may be interpreted "in the sense of Löhnis' symplastic hypothesis, in which case one would expect that a regeneration of the organism from the gran-

ules could be effected." This naturally leads us to a consideration of Löhnis' work. Briefly it may be stated that, as a result of his work and that of his associate (**29, 30**) largely on various species of *Azotobacter*, a number of clear-cut forms have been recognized within a species; among these are the following: first, a roundish coccoid form, second, a rod-like form, third, a form in which the coccoids and rods show granules or gonidia, occasionally taking the form of buds or branches capable of reproducing as such or of giving rise to the parent form, fourth, an amorphous form or symplastic stage in which the contents of coccoids and rods have fused into a more or less irregular mass free from definite cellular structure and possessing the power of producing living granules or regenerative units, fifth, a form resulting from a union of two or more cells, and sixth, a fungoid form of more or less irregular, large, bladder-like or hyphal cells. It is important to note that they have concluded that all species of bacteria have a symplastic stage and possess other stages comparable to those which they observed in *Azotobacter*. Löhnis and Smith's observations have in part been confirmed by Jones (**26**). He also finds in *Azotobacter* that individual cells "may develop reproductive granules or gonidia within their cell plasm, which on disintegration of the mother cell are dispersed, increase in size, become typical *Azotobacter* short rods, ovals or spheres, and reproduce by fission. The young cells are motile. The reproductive granules vary in size, some being very minute." Jones was unable to filter these through a Berkefeld filter. In addition to the gonidia, he is also able to observe the form that Löhnis and Smith have designed as the "symplastic stage" and the regenerative granules which arise from the symplasm. "On emergence from the 'symplasm' these granules grow to young *Azotobacter* cells and reproduce by fission." Just what evidence he used for convincing himself that the granules grew is not stated but it is presumable that the appearance of stained preparations was the criterion. As to the large, irregular, involution (?) forms that Löhnis in part designates as fungoid, Jones also was able to find these, but, contrary to Löhnis and Smith, he finds them to multiply only to a very limited extent, the multiplication being in the form of a budding process. He studied these

in a hanging block culture and "in only a very small percentage of cases did the involution forms show any tendency to develop or reproduce." Finally, he is unable to confirm Löhnis and Smith's findings of spores or of any true conjugation. Jones did not observe the gonidia or the symplastic stage in species of bacteria other than in *Azotobacter*, although, judging from his statements, one may infer that he looked for them in other microbes. One is forced to conclude that Jones' confirmations of Löhnis' work are very slender.

It here becomes desirable to undertake an analysis of Löhnis' work and the proofs that he has presented for the viability of the forms, enumerated previously, that he has found within a species of *Azotobacter* and which he believes are also to be found in all species of bacteria. First of all it should be stated unequivocally that the forms he pictures are not contaminations or artifacts, for the writer's own studies of living, unstained preparations as well as the stained preparations show practically all the types that Löhnis has found and those here shown are in a species quite unrelated to *Azotobacter*. In the days of Alfred Fischer it was perhaps necessary to emphasize the common occurrence of plasmolysis, plasmoptysis and other artifacts in stained preparations, as well as the necessity of guarding against contaminations, but it is hoped that bacteriological knowledge and technic have made some advance since that time. While Löhnis may have had some contaminations in the hundreds of cultures which he worked with, it would be ridiculous to assume that any large part of his work is based on contaminations. It is more reasonable to assume that his cultures were largely pure and that the forms he found belong to the species to which he ascribed them. But, granting this, are his interpretations of the functions of these forms and the role they play in the life cycle open to question? His evidence may roughly be divided into two classes: one, microscopic studies, largely of stained preparations, and two, filtration experiments. As far as his published micro-photographs are concerned, much additional evidence may still be desired. This is particularly true for figures which would clearly show the transition of one stage to another, for example, gonidia giving rise to other forms, or symplasm forming reproductive

bodies. Figures showing arthrospores, cysts, and endospore-formation are far from clear. The photographs as a whole are not convincing. Concerning his filtration experiments, he attempted to pass a number of organisms, which showed gonidia, through Chamberland bougies and was able to see the small gonidia by the use of the dark field, "some of them being actively motile." Large bodies were not observed. The filtrates, when transferred to various media, all produced a very scant, thin, slimy growth, and microscopic observations showed in no case any large forms developing which would be comparable to the normal vegetative forms of the bacteria. Stained preparations of these transplants showed "gonidia germinating to minute rods" and dark-field studies showed "clearly that the filterable gonidia also form a symplasm in the same manner as the larger ones, which in its turn produces new small cells." His photograph of this symplasm is not especially self-revealing; it shows simply a clump of minute particles. When small quantities of filtrates of *B. subtilis*, *Bact. pneumoniae*, and *Bact. fluorescens* were transferred into ammonium-citrate solution, a "quick regeneration took place," with sediment formation in two days' time. Stained preparations of these showed "many pale, stained, small granules and minute rods, . . . and also larger dark stained oval forms 0.5 to 1 μ broad, 0.75 to 1.5 μ long. These forms still differ considerably in their appearance from the normal rods of *B. subtilis* and *Bact. fluorescens*." "That they will turn back entirely to the normal large vegetative cells is not doubted, *but this still remains to be tested experimentally*." The writer has italicized the last clause because this seems to him as representative of the uncertainty of this filtration work. Summarizing the evidence that Löhnis and Smith have presented on the viability of the different forms and their relationship to each other, it may be stated that it falls short of proving their contentions. Their work, and Löhnis' splendid effort of bringing together the literature on the subject, has been very stimulating and may eventually lead to a far clearer understanding of the developmental possibilities within a bacterial species. That the evidence up to date is nearly as complete as some bacteriologists consider it to be (**22**) is very doubtful. For the present the writer is inclined

to believe that most, if not all, of the granules here described and illustrated represent a disintegration process of cells that are about to die and that this death is merely the outcome of autolytic reactions occurring sooner or later in all cultures of microörganisms. He further believes that Löhnis' gonidia and symplastic stages may be placed in the same category but he reserves judgment on certain large, irregular forms, frequently designated as involution stages representing the "lame and the halt" among the bacterial populations. More will be said of these later on.

The next feature of interest that is occasionally observed in stained and in unstained preparations of the organism isolated from potato plugs consists of certain large spiral or whip-like structures (PLATES 32, 33). They are to be observed quite frequently in mounts from the syneraesis water at the base of nutrient agar slants when the bacteria are actively motile and when stained preparations show large numbers of flagella. The writer believes these to be abnormally large flagella representing a group that have fused together and comparable to the teratological specimens that one observes in higher plants and animals, consisting of a fusion of tissues and organs giving rise to various forms of monsters. As observed in stained preparations they vary considerably in size and particularly in shape. Often they appear as rather thin spirals, though always appearing much thicker than normal or average flagella, of equal diameter and tapering but slightly at one or both ends. In this form they bear a very close resemblance to spirochaetes. At other times they take the form of wavy spindles, appearing very thick and heavy towards the middle and gradually tapering down towards the ends, simulating an organized symmetrical body comparable to those seen in the genus *Spirillum*. Then again they may be observed as giant whips taking the form of irregular spirals, being very thick at one end and gradually tapering towards the other end. Usually they are seen unattached to any rod but occasionally they give the appearance of being attached to a rod close to a pole (see PLATE 33). Various investigators have at times observed similar structures and have interpreted them to be clumps of flagella. Gins (**20**) observed them in *B. typhi* and *paratyphi* B. Novy (**37**) found them in condensation water of motile bacteria.

They occurred as single, large, spiral-shaped structures originating from the ends of rods or as "more complicated braided forms not unlike the strands of a rope, usually spindle shaped." Mellon (**32**) in commenting on Novy's observations of these structures concluded that "they were not flagella since they stained with simple dyes. . . . They were not motile." He considers them "the same as the chromatin skein" which he observed within the cells of *B. coli*. While there is the possibility that structures other than flagella may form spiral-like bodies, and Mellon's figures clearly indicate this, yet there can be very little doubt that the bodies described by Gins, by Novy, and those here presented are abnormal flagella, which may consist of single aberrant forms or of groups which have fused together. In view of their relatively enormous sizes it is not surprising that they stain with simple dyes and are non-motile. Indeed, the larger ones are clearly observable in unstained mounts, as previously indicated. The fact that quite a few of them have been observed in the organism under discussion, perhaps more than have previously been recorded as observed in any one species, does not necessarily mean that they are more common in this species than in others, particularly in other organisms where flagella are inclined to be large and present in relatively large numbers, but that the staining method utilized as well as the time spent in studying the morphology of this organism has presented exceptional opportunities of observing them. The writer has little doubt that the same sort of structures will in time be found in most, if not in all, motile bacterial cultures.

Various other forms have been observed in the organism obtained from the potato plugs, including a stage which is quite comparable to Löhnis' symplasm. What the writer has seen consisted of an irregular mass of material which, as a whole, stains rather lightly but with localized parts scattered irregularly through the mass taking a deeper stain. Sometimes these darker staining granules appear with a definite outline, either in the form of a minute roundish or oval-shaped structure, or as a small rod. Mostly, however, they are quite irregular or indefinite in outline, closely resembling the particles which result from the disintegration of the rods, and the writer is inclined to view the whole

structure as a mass of dead matter, in various stages of decomposition or disorganization, fortuitously clumped together, and representing the remnants of the fragmented particles. Sometimes parts of walls or the whole of old walls of rods are mixed in with the material, most of which is interpreted as being the cytoplasmic, storage, and nuclear material together with metabolic biproducts. Another stage to be observed in this organism consists of relatively large cells (PLATE 31, middle figure), usually clinging together in the form of filaments, many of them being bladder-like or spindle-shaped, while others are cylindrical. When seen unstained they closely resemble the hyphae of a fungus mycelium, the individual cells being filled with a compact, granular substance which gives the impression of being reserve food material. They take the anilin stains very readily and usually stain so deeply with carbol-fuchsin that no interior structures are observable. In width they are four to eight times the size of normal, vegetative rods. These mycelioid cells are commonly found in the sediment of old nutrient broth cultures, although they may occasionally be seen in young cultures of both broth and nutrient agar. They have been observed in all the organisms, previously mentioned, in which granules with attached flagella have been found. The writer ventures to predict that they will eventually be observed in most species of bacteria. They have already been observed and illustrated in quite a few species including *B. coli* (**31, 33, 34**), *B. anthracis* (**27**), *B. mallei* (**5**), *B. Chauvoei* (**19**), *Clostridium butyricum* (**38**), and in *Azotobacter* (**29**, fig. 20). Löhnis interprets some of these bodies to be spores (**28**, p. 65) and others gonidangia (p. 124, 125), that is, bodies capable of reproducing a number of gonidia. There is no reason to doubt that they have frequently been observed by bacteriologists and taken to be degenerate or involution forms. Whether this is always a true interpretation remains to be determined.

SUMMARY

In discussing variability in bacteria attention is called to the fact that conditions which made for so-called normal growth often preclude the possibility of obtaining other structures which may develop in the same species under more natural conditions.

It is pointed out that variations may occur not only because of different phases in a life cycle, each with its own set of forms and functions, but also because of racial or clonal differences within a species.

While using a flagella stain on a peritrichous, spore-forming organism, originally isolated from a mosaic infected Irish potato tuber, numerous stained particles were observed, and here pictured, in young and old cultures. These particles frequently possessed flagella.

Similar particles were found within the confines of regular rods, often with flagella subtended or oriented toward such particles.

They vary greatly in size and shape, the tiniest approaching the size of the particles constituting the "contagium vivum fluidum" of tobacco mosaic.

Similar particles, but not as abundant, have been observed in *B. typhi*, *B. vulgatus*, *B. mesentericus*, *B. subtilis*, and *Proteus vulgaris*.

Attempts are made to compare these to gonidia, buds, and reproductive granules described by Löhnis and others.

Three different lines of evidence are detailed for determining the viability of the particles, including direct microscopic observation of growth or reproduction, ultra-filtration experiments, and pure culture isolations of particles.

It is concluded that the evidence for viability is uncertain, the ultra-filtration work favoring the theory that the particles are alive while the other lines of evidence are mostly negative.

The writer is inclined to the belief that the particles represent a disintegration process in the course of ordinary autolytic changes.

Various types of spirals are described and figured, some being observable in unstained preparations that are taken to be abnormal flagella.

A description of giant, mycelioid cells is given and the bodies compared with those previously described in a large number of bacteria.

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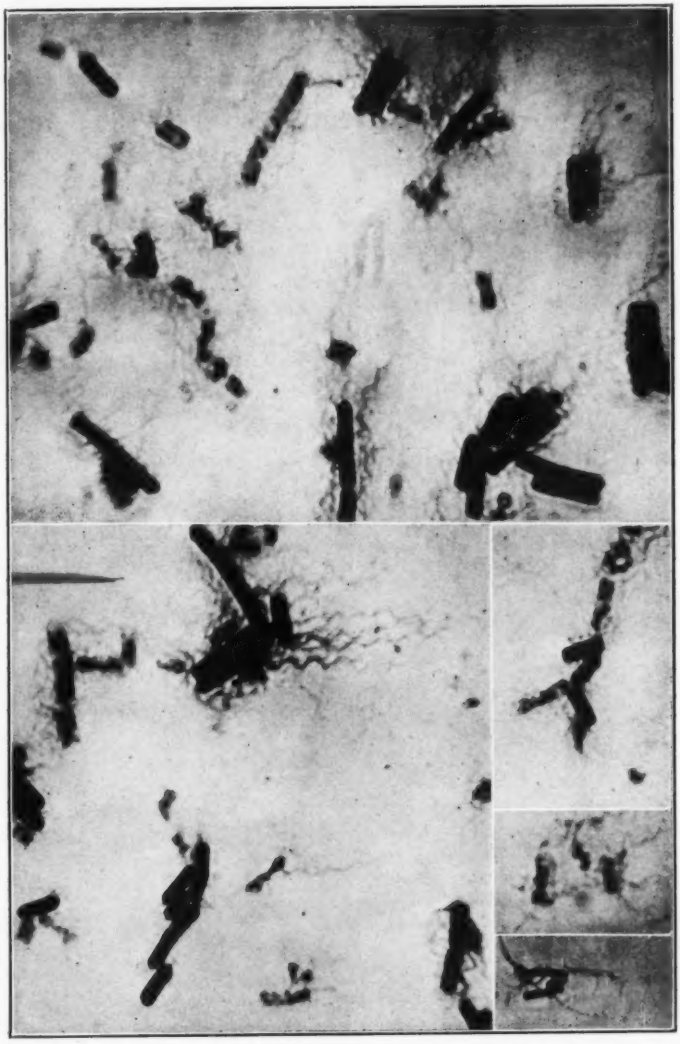

VARIATION IN BACTERIA

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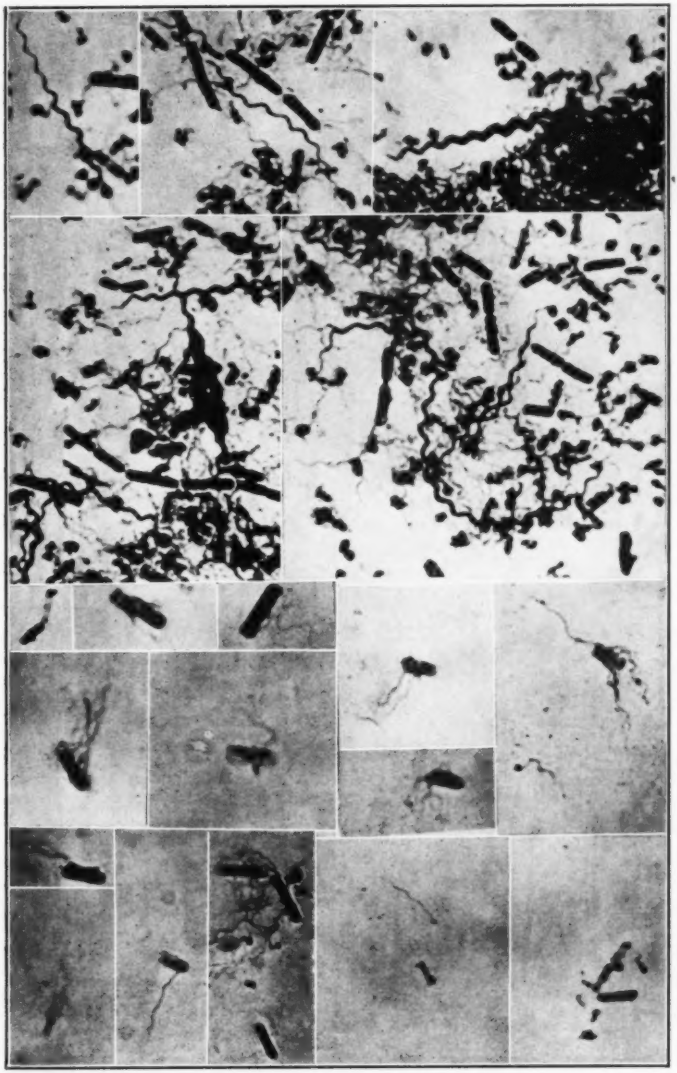

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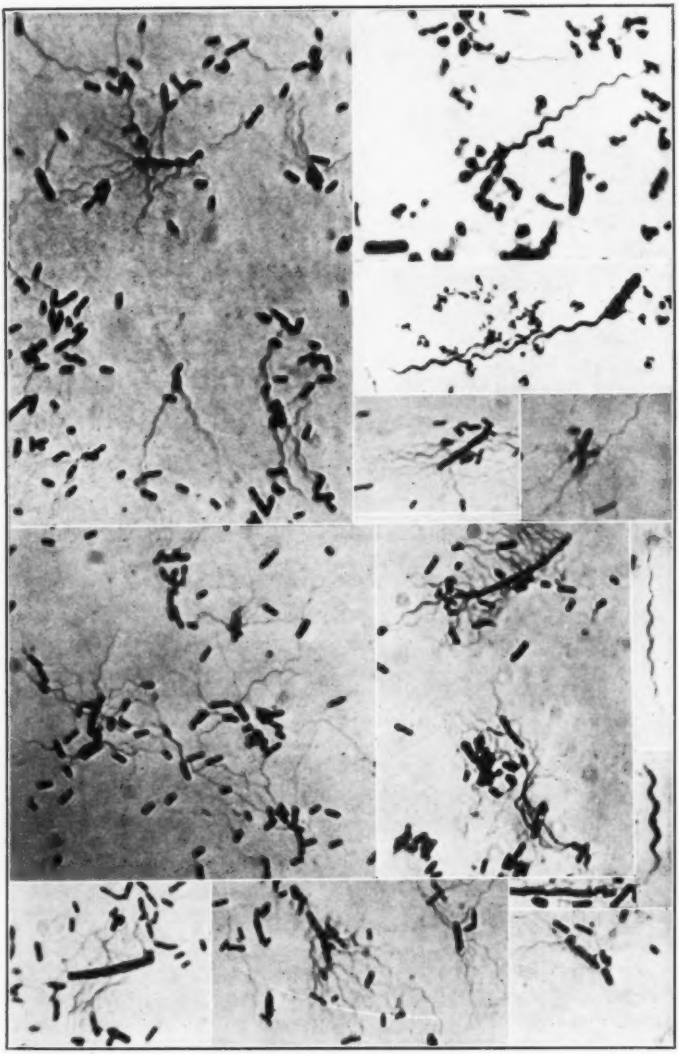

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EXPLANATION OF PLATES

PLATE 29

Upper left-hand figure—colonies of the bacillus isolated from a mosaic diseased tuber, 48 hours old on nutrient agar. Spreading colonies near the glass; surface colonies smaller and with more of an irregular margin. Remaining figures showing fragmenting rods of this organism stained by the writer's flagella staining method. Magnified about 2500 times.

PLATE 30

Rods of the organism isolated from a potato in various stages of disintegration; unfragmented ones are also seen, appearing homogeneous and heavily stained; magnified about 2500 times. Lowermost right-hand figure representing *B. typhi* showing a bud-like projection; magnified about 1500 times.

PLATE 31

Upper right-hand figure showing rods of the potato bacillus with particles in the interior; note a disintegrating rod, toward the middle, with flagella concisely oriented toward two heavily staining particles.

Upper left-hand figure showing mostly remnants of old walls and residuum of interiors of rods, corresponding somewhat to Löhnis' symplasm.

Middle left-hand figure showing mycelioid types of cells of the same organism illustrated in Plates 29, 30 and 31.

Middle right-hand figure showing a localization of cell contents into a definite pattern in the rod toward the center, while surrounding rods are in the process of disintegration. Magnified about 2500 times.

Lower figures show free particles of the same bacillus, with subtending flagella.

PLATE 32

Upper half of the plate shows particles observed within rods of the potato organism looking at times like buds and giving an irregular contour to the rods. Note also free particles with flagella attached. Some of these particles measure less than 0.1 μ. Magnified about 2500 times.

Lower half of plate presents figures of spirals and giant whips stained by the writer's flagella staining method, taken to be abnormal flagella. Magnified about 1500 times.

PLATE 33

Two upper right-hand figures as well as the two narrow middle right-hand figures show various types of spirals of the potato bacillus. Remaining figures represent pure, authentic cultures of *Proteus vulgaris* showing flagella and a granulation process, particularly in the large multiflagellated organisms; magnified about 1500 times.

TEMPERATURE AND MOISTURE RELATIONS OF FOMES ROSEUS AND TRAMETES SUBROSEA

WALTER H. SNELL, W. G. HUTCHINSON, AND K. H. N. NEWTON

(WITH PLATE 34 AND 2 TEXT FIGURES)

Much of the material of this paper was put in form a few years ago as a contribution to the taxonomy of the two polypores with the rose-colored hymenium, *Fomes roseus* (Alb. and Schw.) Cooke and *Trametes subrosea* Weir. Until quite recently these two wood destroyers were commonly called *Fomes roseus*, with perhaps a mental reservation that there might really be two forms. Overholts in 1915 stated that the two forms were quite distinct, however (**6**, p. 68). The senior writer had become convinced of the same fact both from collecting these forms and from observations of cultural behavior in the laboratory and fructification in the field. In connection with various studies of structural-timber-destroying fungi, the senior writer became interested in means of identification of the cultures of wood-destroying fungi and especially in the thermal responses of the mycelium in culture as a feasible means of differentiating many of them (**8**, p. 24). With a view to adding material in substantiation of Overholts' separation of these two forms, work was done on various pairs of closely related polypores as well as these two, in order to find out the reliability of temperature responses as a differential. About the time that the material was ready for publication, Weir's paper (**12**) clearing up the situation with regard to these two fungi appeared and the pertinence of the temperature reaction studies lost its immediacy. In view of this fact, the material was withheld for the addition of more complete material on the moisture relations than was then at hand.

VALUE OF THE TEMPERATURE FACTOR IN IDENTIFICATION OF CULTURES OF WOOD-DESTROYING FUNGI

It is often desirable to be able to determine the identity of wood-destroying fungi in the absence of fructifications. This identifica-

tion is possible in many cases because of the association of a definite and characteristic type of decay with the presence of a certain organism. There are, however, groups of wood-destroying fungi the individuals of which produce decays so nearly alike that it is difficult to distinguish them. This is true of the decays caused by some of the commoner forms, by many closely related species, and by many of the important structural-timber-destroying fungi. If, in addition to the absence of fructifications, certain distinctive formations such as strands, colored mycelium, etc., are not present, the only remaining method of determination is by means of cultures.

Falck in Europe (**1** and **2**) has done considerable work in distinguishing the decays of certain structural-timber-destroying fungi by means of such manifestations as mentioned, but has not developed the use of cultures for the same purposes. Cultural studies have received more attention in this country. For the purpose of distinguishing between species, even closely related ones, Long and Harsch (**5**) emphasize the advantages of color reactions obtainable upon a series of several agars selected because of their value in yielding differential color effects. The senior writer, in making a key of the cultural characters of five mill fungi (**8**, p. 24), used not only the type of growth upon a single agar,[1] color of the growth and presence or absence of the different kinds of secondary spores, but also the comparative rates of growth of the mycelium at certain temperatures.

This temperature reaction was found to be a valuable criterion in the distinguishing and determining of these fungi in culture. The test was successfully applied to certain cultures in the Forest Products Laboratory collection—cultures taken from decayed structural timbers in buildings in various parts of the country the identity of which were known, unknown, or uncertain. The temperature test was also applied to certain pairs of readily distinguishable but closely related species in order to find out if the difference in thermal response of the mycelium of the closely related fungi is the rule or the exception.

One pair of closely related polypores used was *Lenzites sepiaria* Fries and *Trametes protracta* Fries. They are usually considered

[1] Malt agar—3 per cent agar, 2½ per cent malt (Trommer's diastasic extract).

as separate species although it has been suggested more than once that *Trametes protracta* is only a poroid form of the former. Point is often given to this suggestion by the occasional occurrence of the two species or forms near together, even on the same piece of wood. The senior writer has found them together several times on bridge timbers. On one occasion, on a rough log bridge over a mountain stream, the senior writer found three of four poroid *Trametes protracta* forms occurring among a hundred or two lamellate *Lenzites sepiaria* fruit bodies. On another bridge of sawn timbers, the numbers of fruit bodies of the two species were more nearly even. Inasmuch as two pairs of cultures of each fungus (one pair of single spore cultures and one pair of tissue cultures) were at hand, a complete set of tests was run. The tests were made in triplicate and often repeated at all temperatures, upon agar from the same batch. The agar plates were poured from tubes containing 20 cc. of the medium. The plates to be used for the tests were inoculated with a 1 cm. square block taken from the growing border of a plate culture of the fungus, the square of inoculum being placed mycelium side down on the inoculated agar. The measurements recorded in Figure 1 are the results of averages of radial growth from the four sides of the inoculum square on the triplicate plates. Plates showing irregular growth for any reason were discarded, and the test was repeated. Very seldom, there was almost no growth from one side of the inoculum, whatever the reason might be, but as a rule the growths were very regular and there was almost no variation in the 12 measurements taken. Two points on the curves in the figures represent the extremes of variation.

It is to be noted from these tests that there is no apparent difference in the optima of the two species. *Trametes protracta*, like *Lenzites sepiaria*, has a comparatively high optimum temperature—between 30° C. and 34° C. Their upper limits of growth are, however, different. *Lenzites sepiaria* is not inhibited until after 40° C. is reached, while *Trametes protracta* was only barely growing at 38° C. and would not grow at all at 40° C. Also, the rates of growth of the two organisms are quite different. *Lenzites sepiaria* grows faster at all temperatures, the difference being more pronounced between 28° C. and 36° C.

There is a pronounced difference in the temperature reactions of the two organisms, not as to optimum but as to rate of growth, except at the lower temperatures. This fact supports the more commonly accepted view that *Trametes protracta* is a species distinct from *Lenzites sepiaria*. A test of growth upon a single agar at temperatures from 30° C. to 36° C. would serve to distinguish the fungi in culture.

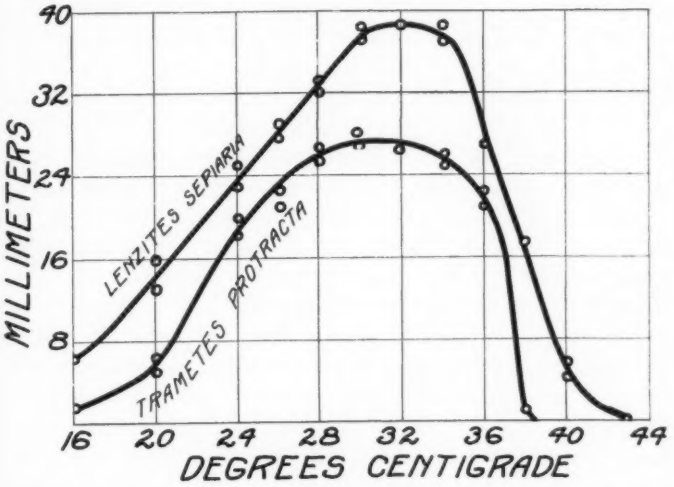

FIG. 1. Smoothed curves of the growth of the mycelium of *Lenzites sepiaria* and *Trametes protracta* on malt agar in relation to temperature. The pairs of circles at the different temperatures represent limits of variation of growth.

A second pair of species tried out in this way was *Polyporus abietinus* Dickson ex Fries and *Polyporus pargamenus* Fries. The results here were not as striking as with the preceding pair, but the two curves were sufficiently different in a number of places to provide a good differential on the basis of temperature, if a differentiation of the cultures of the two fungi should be desirable.

A third pair tried included *Polyporus resinosus* Schrader ex Fries and *Polyporus benzoinus* Wahl ex Fries. As a matter of convenience here, the two forms are considered to be separate species, although it is recognized that there is some dispute concerning the propriety of this arrangement. Many, including

Overholts (**6**, pp. 47 and 52), believe that the latter is only the coniferous wood form of the former species. Others, as for instance Lloyd (**4**, p. 334), think they are quite distinct and distinguishable. The senior writer has been interested in attempting to determine this matter. The most promising experiments embrace inoculations of hemlock, basswood and maple logs and bolts with cultures of each of the species, which have been made in two localities in the Adirondacks (experiments now 3 years old). The temperature test was applied also in order to see what it might contribute. Without presenting the data here, it may be said that the curves of growth are nearly the same for both fungi at most of the temperatures, but that there is a difference at 24° C. It may be added also that there tends to be greater irregularity in the growth of *Polyporus benzoinus* at 24° C. than in that of any other fungus at even the extreme temperatures.

This test was also applied in an earlier piece of work (**9**, p. 162) to demonstrate that the Agaric causing decay in cotton mill roofs is *Lentinus lepideus* Fries and not *Lentinus tigrinus* Fries. The growth of the latter at 30° C. was nearly double that of the former and the difference was useful in substantiating the less constant differences in appearance of cultures.

It is seen that the thermal responses of the mycelium offer reasonably definite means of separating the cultures of species that are ordinarily distinguishable on morphological grounds. This is shown by the positive results of the tests upon three sets of recognizable species, and further supported by lack of definite evidence in the tests upon *P. resinosus* and *P. benzoinus*, two forms about which there is more dispute from a mycological point of view, as they are at present separated chiefly on the basis of host.

With regard to the possibility of settling disputes as to the specificity of closely related forms, the foregoing experiments offer some encouragement, but no definite assurance. It is seen that well-defined species may react differently thermally. On the other hand, there seems to be no reason why they of necessity must. If *P. resinosus* and *P. benzoinus* are really two different species, the slight and irregular differences in their temperature curves give only slight evidence of the difference, very little upon

which to place any dependence. Leonian (**3**, p. 452) found that there was no specific difference in the rates of growth of the species of *Phytophthora*. While for the most part, heretofore, differences in physiological behavior have been taken only as evidence of varietal or racial difference, there is a growing tendency to depend upon functional response as a factor in taxonomy and in phylogeny. It would seem that organisms that live differently are inherently different, although difference in a single respect would hardly be sufficient ground for making species.

TEMPERATURE RELATIONS OF *Fomes roseus* (ALB. & SCHW.) COOKE, *Trametes subrosea* WEIR AND *Trametes Feei* FRIES

Working originally with the idea that, if there was a definite difference in temperature response, it might indicate a difference of species and would at least support other evidence, a rather extensive set of tests was run with cultures of *Fomes roseus* and *Trametes subrosea*. In view of Weir's paper (loc. cit.) to the effect that there are distinct morphological differences between the two forms, the following results merely serve to substantiate his conclusions and to show the possibility of the use of similar methods with other species.

Trametes Feei was added upon Weir's suggestion merely as a matter of interest in connection with the entire problem and he very kindly sent a fruit body for culture purposes. Thus there are included the three polypores in the United States that have a rose-colored hymenium.

The cultures used were the following:

1. *Trametes subrosea*—single spore culture from tamarack, Wisconsin, 1916.
2. *Trametes subrosea*—single spore culture from *Prunus* sp., Rush Lake, Minn., 1917.
3. *Trametes subrosea*—single spore culture from spruce log, Crawfords, N. H., 1919.
4. *Trametes subrosea*—tissue culture from fruit body on spruce pulp bolt from Canada, 1921.
5. *Trametes subrosea*—tissue culture from fruit body on red oak fence post, Jackson, N. H., 1922.
6. *Trametes subrosea*—tissue culture from fruit body, Warrensburg, N. Y., 1923.
7. *Trametes subrosea*—single spore culture from same as 5.
8. *Fomes roseus*—tissue culture from fruit body on spruce beam, Jeffersonville, Vt., 1919.

9. *Fomes roseus*—tissue culture from fruit body on spruce beam, Jackson, N. H., 1922.
10. *Fomes roseus*—tissue culture from fruit body on hemlock plank, North Conway, N. H., 1922.
11. *Fomes roseus*—tissue culture from fruit body on Douglas fir down log, Big Basin, Calif., 1923. Sent by Dr. E. P. Meinecke.
12. *Fomes roseus*—tissue culture from spruce (?) beam on ground, Warrensburg, N. Y., 1924.
13. *Trametes Feei*—tissue culture from fruit body from Florida sent by Weir, 1924. Other data lacking.

The significant results are shown in Figure 2. Curves of all the cultures in the above list are not shown for various reasons.

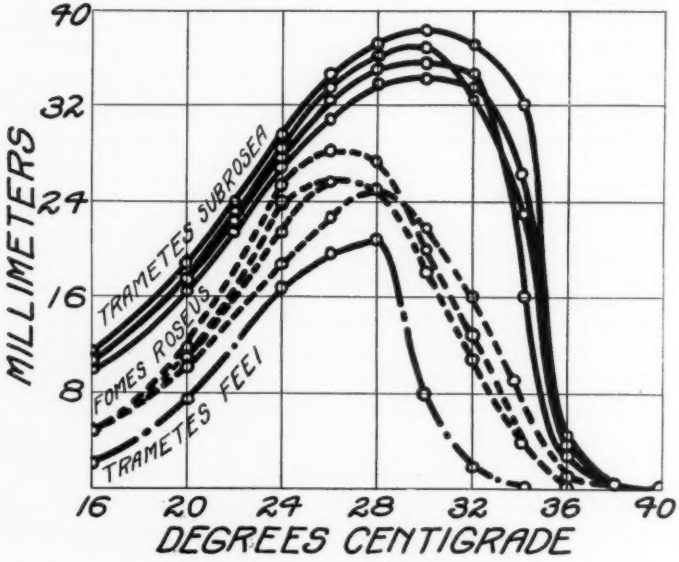

FIG. 2. Smoothed curves of the growth of the mycelium of *Trametes subrosea*, *Fomes roseus* and *Trametes Feei* on malt agar in relation to temperature. The circles represent averages of radial growth from the inoculum block on triplicate plates, many times repeated. The cultures represented by the curves are as follows: *Trametes subrosea*, proceeding from the top down along the 32° C. line—1, two cultures from red oak (nos. 5 and 7 in Table I); 2, from spruce (no. 4); 3, from conifer (no. 6); 4, from cherry (no. 2); 5, from tamarack and spruce (nos. 1 and 3). *Fomes roseus*, from top down, along 24° C. line—1, from spruce (no. 9); 2, from spruce and hemlock (8 and 10); 3, from spruce (?) (no. 12); 4, from Douglas fir, California (no. 11).

In the first place, it was found that there was no difference in the curves of single spore and tissue cultures from the same fruit body. In the second place, there is no point in showing all the curves that fall in between the outside limits for a species, or in presenting pairs that nearly coincide. All the *Fomes roseus* curves are shown, but of those of *Trametes subrosea* only the more interesting are given. These include two curves of cultures of species growing on coniferous woods and two on deciduous hosts.

There is but one curve for *Trametes Feei*. Nothing is known about any variations within this species, but on the face of the only available result this species grows more slowly at all of the temperatures and is inhibited from growing at a lower temperature. It could be differentiated from the other two species if grown from 30° to 34° C.

Leaving aside for the moment the individual variations in the different cultures of *Fomes roseus* and *Trametes subrosea*, it is seen that there is a difference in the tendencies of the curves for the two species, from 26° C. to 36° C. A test at 30° C. or 32° C. apparently differentiates these two species with absolute reliability. The temperature test is the most reliable way of separating the cultures of the two species, because other characters of the growth upon agar vary so much. In color, the culture from spruce at Crawfords, N. H., is always pure white. The one from oak is a little pinkish (La France pink [1]), and the one from cherry is streaked with old rose or jasper pink, and may be somewhat Pompeian red with streaks of hazel. This latter culture was not much different in appearance from the *Fomes roseus* culture from California, although the other cultures of this species are decidedly dark colored—hazel to pecan brown or Rood's brown.

The consistency of the mycelial mats is likewise variable. The Crawfords culture of *Trametes subrosea* is decidedly "bunchy" tomentose like washed cotton flannel, and powdery when worked with a needle, while the New England *Fomes roseus* cultures form a very tough mycelial skin on the surface of the agar. The consistency of mycelial mats of the other cultures varies between these two extremes, the skin being tougher the more pronounced the color.

[1] Ridgway, Robert, Color Standards and Color Nomenclature, 1912.

An interesting example illustrates the reliability of the temperature test in differentiating the cultures of *Fomes roseus* and *Trametes subrosea*. In the earlier work, before many different cultures had been tried, Dr. Meinecke's specimen from California was welcomed as an opportunity to test the conclusions formed up to that time. The plant appeared without any doubt to be what was then called *Trametes carnea* by those who admitted of a difference in the two forms. As "*Trametes carnea*" it was tested. The first results were so diasppointing as to appear to threaten the entire study. At 24° and 28°, the results not only did not coincide with what was expected of "*Trametes carnea*," but were even short of what had been obtained for *Fomes roseus*. The growth was from 3 to 8 mm. less at those temperatures than for *Fomes roseus*, whereas up to that time "*Trametes carnea*" had always given 2 to 8 mm. more of growth at those temperatures.

When the complete curve had been obtained, it was obvious that the plant from California was *Fomes roseus* and not "*Trametes carnea*" (*T. subrosea*). About this time, Weir's article already mentioned appeared, and while the writers were unable to satisfy themselves as to the identity of the fungus on the criteria given in the key, on the other hand Weir wrote of the specimen that he "unhesitatingly called it *Fomes roseus*."

At about the same time, it was noticed also in the cultures of *Trametes subrosea* that the one from oak had a consistently greater growth at most of the temperatures, and with very little variation in the different runs. In fact, this culture was found to respond more regularly than any other culture that has been tried. It was thereafter found that curves plotted for the various cultures of both species were different in some cases, although of course some curves coincided. The culture of the aforementioned species showing the least growth at most of the temperatures happens to be the one from another hardwood—the one obtained from the exposed heartwood of a living cherry tree. The cultures from what is considered the normal substrate for *Trametes subrosea*—wood of coniferous trees—gave a more nearly uniform response. The natural human tendency to endeavor to explain everything, even upon quite gratuitous assumptions, calls up two

suggestions in this connection: one, that the physiology of the plant has been changed slightly by the somewhat unusual food substrate—*i.e.*, angiospermous wood; the other, that there may be different strains or forms of the organism, some of which have become adapted to hardwood habitation.

There is no reason why there should not be strains of these wood-destroying fungi. Schmitz (**7**) has found what appear to be strains of *Fomes pinicola* Swendener ex Cooke. Further work to obtain data upon this point in connection with the fungi considered in this paper is contemplated.

OBSERVATIONS ON THE MOISTURE RELATIONS OF *Fomes roseus* AND *Trametes subrosea*

The senior writer has been interested for some years in various aspects of the moisture relations of some of the wood-destroying fungi and certain fungi producing cankers on woody plants (cf. **10** and **11**). There is much of interest and of importance in the ecology of the structural-timber-destroying fungi. Moisture appears to be the determining factor in the occurrence of certain of these fungi. In connection with general observations on the two fungi under discussion over several years, it has been noted that *Fomes roseus* has been found with few exceptions upon hewn timbers more or less exposed to drying by sun or wind—at least in situations essentially not moist. On the other hand, *Trametes subrosea* has been collected on logs covered with bark or on wood in situations decidedly moist. The former has been located in fields, old sawmills and other buildings or very open woods; the latter in ravines, near brooks, waterfalls, etc., or if in open locations, well protected by grass, ferns or other plants.

In a pasture which was once the well-known Enchanted Woods (white pine) near North Conway, N. H., surrounded by younger pine growth, there was found a scattering of these two species of polypores that provided a chance for observation and experiment with regard to their moisture reactions. In the clearing there stood, up to recently, the framework of the old sawmill that cut up the pine of this beautiful spot. Among the various fungi found in this old sawmill were live sporophores of *Fomes roseus*, occurring on pine beams in some of the moister and more protected

places overhead, in the drier places on the framework and on the pine beams on the ground outside, exposed to sun and wind. There was no *Trametes subrosea* on the pine or hemlock wood of the structure at all, but it was found in abundance along with *Fomes roseus* in a pile of hemlock, spruce, and pine logs and bolts within a hundred feet of the sawmill. This pile of logs and bolts was backed up to the east side of a stand of sapling white pine, and surrounded with a dense growth of sweet fern which reached up 15 or 20 inches on the pile. Here there was a sharp separation in the occurrence of these two forms: *Fomes roseus* on worked timbers in the open and on decorticated bolts on the top of the pile—both situations well ventilated; and *Trametes subrosea* on unworked timber in moist log-pile conditions, down low and protected from drying by the ferns and grass. This sharp separation of habitat suggested a difference in moisture requirements or dryness tolerances of the two fungi, either with regard to vegetative growth within the wood or to fructification on the outside. It appeared that the habitat of *Fomes roseus* was drier both as to substrate and as to relative humidity of the atmosphere than that of *Trametes subrosea*. The timbers upon which the former fungus grew should have been much drier not only by reason of their more open situation exposed to ventilation, but also because most of them were barked and sawn or hewn, and therefore more readily dried after each wetting. On the other hand, the logs in the piles bearing the latter fungus, with or without the bark, not only dried out less rapidly, but were not so well ventilated. Whatever may be the effect of the sawing or hewing of timber upon the rapidity of its drying, the effect of the bark is definite.

No tests were made of the moisture content of the logs or bolts in the pile bearing the two fungi, or of the beams in the mill bearing only *Fomes roseus*, but it was of course obvious that the upper bolts in the pile bearing only this fungus were dry on the outside most of the time except shortly after rains, while the lower bolts and logs bearing *Trametes subrosea* were wet longer, if not all day at times. However, the moisture content of the interior of the logs without doubt varied with that of the exterior.

In order to gain information upon the difference in relative

humidity of the atmosphere in these two places where the two fungi fruited, many readings were taken during the summer of 1922 with a hygrometer, at different points in both the log-pile and the old sawmill. The readings were taken repeatedly at all times of the day and night, and under all conditions of weather. A few of the readings selected at random are given in Table I.

TABLE I

RELATIVE HUMIDITY CONDITIONS WHERE *Fomes roseus* AND *Trametes subrosea* FRUITED, NORTH CONWAY, N. H., 1922

Day and time	Weather	Relative humidities			
		Bottom of log-pile where *Tr. subrosea* fruited	Top of log-pile where *F. roseus* fruited, but no *Tr. subrosea*	Sawmill where *F. roseus* fruited	Beam outside of sawmill where *F. roseus* fruited
August					
4th— 5 P.M.	Sunny, windy	68%	58%	49–57%	49–50%
7th— 9 A.M.	Rain	93%	90%	90–93%	90–93%
7th—12 M.	Sun and wind after rain	84%	78%	82% (floor overhead soaked)	74%
9th—12 M.	After 2 days of rain	60%	50%	50% (*F. roseus* making new growth)	48%
16th—all day[1]	Hot, bright, little wind	48–53%	41%	43–46%	39–41%
18th—all day[1]	Hot, bright, little wind	71%	67%	68%	66%
21st—all day[1]	Bright, cool, high wind	45–48%	39–40%	43–44%	35–38%
26th—all day[1]	Following rain wind rising	77–81%	72%	72–77%	74%
27th—all day[1]	Bright	77–81%	71%	69%	60%

Several points may be noted from the preceding table with respect to the atmospheric humidity at the four locations:

1. Only on rainy days was the relative humidity *in* the sawmill, *outside* the sawmill, and at the top of the log-pile where *Fomes roseus* fruited as high as that in the log-pile where *Trametes subrosea* fruited.

[1] Several observations between 9 A.M. and 6 P.M.

2. The relative humidity in or near the sawmill and on the top of the log-pile was never higher than that down in the log-pile.

3. The relative humidity in the sawmill where *Fomes roseus* fruited was about the same as that upon the top of the log-pile where this fungus fruited also, and where *Trametes subrosea* did not fruit (and apparently could not fruit, as is shown below).

4. The relative humidity in the sawmill was 6 per cent to 10 per cent lower most of the time than that inside the log-pile or down in the shelter of the sweet fern where the *Trametes subrosea* fruited.

5. The relative humidity near the beam outside the sawmill with the *Fomes roseus* was drier at all times than the sawmill.

As to the fruiting of these two species at least, it would appear that *Fomes roseus* could tolerate somewhat drier atmospheric conditions than *Trametes subrosea*. Tests were made in August, 1922, to check these conclusions, by changing the places of the bolts bearing the two species respectively. Bolts on the top of the pile bearing the former species were placed down at the bottom, in the protection of the sweet fern, and bolts from down in the protection of the sweet fern bearing the latter species were placed on top in the open. The expected happened. The sporophores of *Trametes subrosea* (transferred from the bottom to the top) immediately dried up, became rigid instead of rubbery, revived during the next rain and then dried up to revive no more. No change was noted that year in the *Fomes roseus* placed down low among sweet fern.

When these bolts were examined the next fall (1923), *Trametes subrosea* fruit bodies had appeared alongside of the *Fomes roseus* on the bolts which had been transferred from the top to the bottom of the pile and the latter fungus seemed to be fruiting normally. Identification of these forms was made morphologically and culturally. On the bolts on the top of the pile, the dead *Trametes subrosea* fructifications of the previous year were still there, and also a few inches away on two bolts was a new fruit body one season old. These appeared to be *Fomes roseus*. One half of each was cut off for study and the other half of each left for further observation. Cultures made from each of these halves run at 30° C. proved the species to be *Fomes roseus*.

That same fall (1923), the bolts which had been removed from the top of the pile to the bottom in 1922 with the changes noted and upon which *Trametes subrosea* had now fruited in the moister conditions, were changed back to the top again. The other bolts brought from the bottom to the top which had developed *Fomes roseus* were left on the top. When all these were examined in the fall of 1924, it was found: that the halved fruit bodies of *Fomes roseus* had grown another layer of tubes, substantiating the culture test; that no *Trametes subrosea* had developed on these exposed bolts in two years alongside the ones which had been there previously and died, and that the bolts which in 1922 had had no *Trametes subrosea* on top of the pile, but had developed some of these fruit bodies the next year down at the bottom of the pile, now had only the dried fruit bodies of this species, while the original *Fomes roseus* was continuing growth.

This working theory that *Fomes roseus* was better adapted for living and fruiting upon dry hewn timbers than was *Trametes subrosea* and would be found more often in drier places was vindicated many times in the White Mountains through several summers.

This same difference in fruiting was noted also in wood block cultures in flasks (PLATE 34). *Fomes roseus* always fruited higher in the flask where it was drier. The arrow "A" at the *Fomes roseus* flask shows the level up to which fruit bodies grew in considerable abundance, the normal limit in flasks, although occasionally a few are formed higher than this. The lower arrow "C" near the "*Trametes carnea*" flask shows the normal upper limit of sporophore production by this fungus in flasks; the upper arrow "B" shows the absolute limit, which was approached only occasionally and then only in small numbers. In this particular flask, the sporophore showing at this level is the only one in the flask, and in fact was the only one that high in a dozen or more flasks of that series.

SUMMARY

The value of temperature responses of the mycelium as a means of differentiating cultures of wood-destroying fungi is elaborated. A few pairs of closely related fungi are used to illustrate the feasibility of using this reaction.

The temperature test is applied for the purpose of distinguishing *Fomes roseus* (Alb. & Schw.) Cooke and *Trametes subrosea* Weir, and also as contributory evidence of their specific difference.

Trametes Feei Fries, the only other polypore in this country with a rose-colored hymenium, was also included in these tests as a matter of interest.

Data are adduced to show that *Fomes roseus* and *Trametes subrosea* are different also in their moisture requirements, or dryness tolerances, with regard to fruiting if not to growth. This was shown to be true not only by observation and experiment in the field, but also in wood block cultures in the laboratory.

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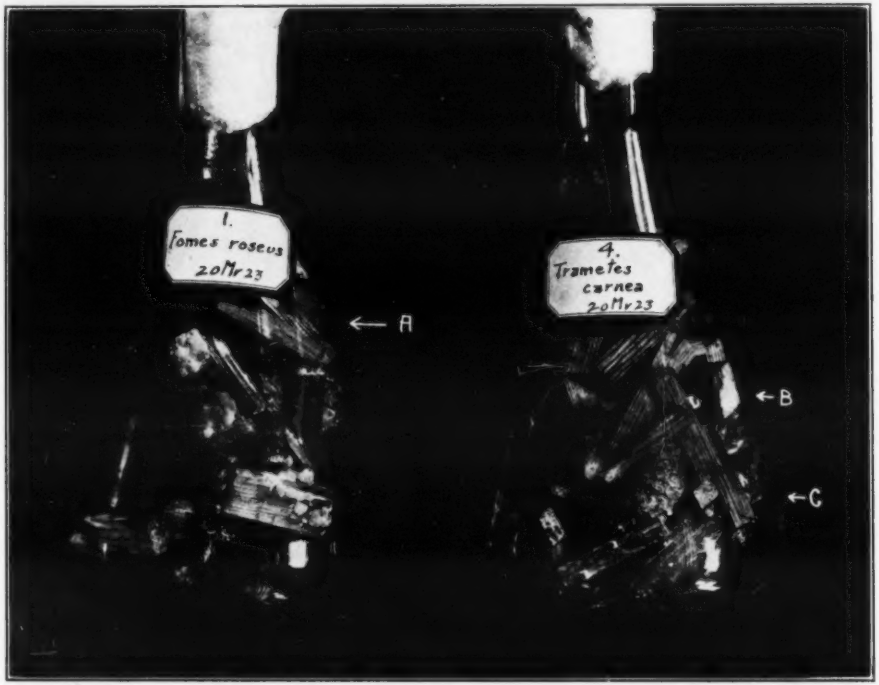

FOMES AND TRAMETES

12. **Weir, J. R.** *Fomes roseus* (A. & S.) Cooke and *Trametes subrosea* nom. novum. Rhodora **25**: 214–220. 1923.

EXPLANATION OF PLATE 34

Sporophore production by *Fomes roseus* and *Trametes subrosea* (*T. carnea*) on wood blocks in flasks, in relation to relative humidity of the air.

It is shown that the fructification under flask conditions by *Trametes subrosea* is confined for the most part to the lower portion of the flask where it is moister (arrow "C"), with only occasional fruiting higher where it is somewhat drier (arrow "B"), and that *Fomes roseus* normally fruits in abundance as high in flasks as arrow "A," where it is considerably drier. Cultures 9 months old. The arrows refer to levels projected horizontally to the front of the flasks (middle of the flasks in the photograph).

STUDIES ON SOME CALIFORNIA FUNGI

LEE BONAR

(WITH 2 TEXT FIGURES)

LASIOBOTRYS AFFINIS Hark.

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On *Lonicera hispidula* Dudl. var. *californica* Jepson.

Found commonly on the above named host in the Coast Range of western California. A specimen of the type collection from Mt. Tamalpais is deposited in the Herbarium of the Cal. Acad. of Sci.

Ellis listed this plant as synonymous with *Lasiobotrys Lonicerae* Kunze, but it was shown by Thiessen to be very different from *Lasiobotrys Lonicerae*. He examined some of the type material and gave a somewhat fuller description of the plant and some figures. A comparison of his figures with those for *L. Lonicerae* readily points out the striking differences between the two plants.

Von Höhnel discussed at length the structure and taxonomic position of the genus *Lasiobotrys*, being of the opinion that it should be placed in the Dothideales. He was handling, for his American material, that distributed by Ellis as *Lasiobotrys Lonicerae* from *Symphoriocarpus*. This has been shown by Sydow to be a true Dothideaceous fungus and named *Rhizogone Symphoriocarpi*.

Von Höhnel listed the imperfect stages of the genus, so far as any were known, under the genus *Kabatia* of Bubak. These had been referred to *Labrella* by Desm. and to *Leptothyrium* by Saccardo. These forms have been assumed to be the imperfect stages because they are found constantly associated with *Lasiobotrys* on *Lonicera* species.

I have collected *L. affinis* many times and at all seasons of the year in California and have not seen any evidence of such, or any

other forms of Imperfecti, associated with the infection on *Lonicera*.

The ascospores of *L. affinis* germinate rather slowly in water. Isolations of single spores have been made and the plant grown in the laboratory for a period of two years. The mycelium is brown to black, in the culture, and forms a scant aërial growth, over the medium which becomes black in appearance. Conidiophores soon form on the mycelium. They are erect dark hyphae and bear a conidium at the tip. The conidium is at first 1-celled and sub-hyaline. The conidiophore continues to grow from below the conidium so that it is pushed aside and then another conidium forms at the tip. This continues until three or four conidia are formed, in many cases, on a single conidiophore. The conidia remain attached and continue to grow, becoming several celled, and the walls covered with tubercular projections and brownish black. These spores when mature are globoid to ellipsoid, with rough wall, muriform, 12–15 microns in diameter and up to 25 microns in length (see Figure 1).

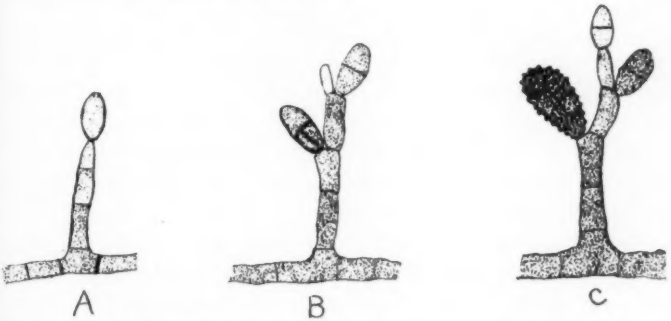

FIG. 1. *Lasiobotrys affinis* Hark. A–C showing stages in the development of the conidiophores and conidia

Long-continued cultivation of the fungus on a wide variety of media and under varying conditions has failed to give anything other than this hyphomycetous form.

I have not seen this conidial form on the host plant. Very clear evidence is here afforded that *Lasiobotrys affinis* Hark. is distinct from other species of this genus listed on *Lonicera* and the following amended description is offered.

LASIOBOTRYS AFFINIS Hark.

Spots scattered, amphigenous, subcuticular in origin, forming black spots which are rather elevated, slightly convex, and 2–10 mm. in diameter. These consist of a thin black hypostroma, from which rise flat-topped columns of sclerotial material. The perithecia nestle free between these sclerotial masses which are slightly higher than the perithecia. Perithecia globose, chestnut brown, 70–100 microns in diameter, few stiff hairs as outgrowths from the perithecial wall. Asci short-stipitate, nearly cylindric, 50–70 × 12–15 microns. Spores ellipsoid to fusoid, very unequally 2-celled, light brown in color, and 14–17 × 6–8 microns. No paraphyses.

Dothidella castanicola comb. nov.

(*Phyllosticta castanicola*) Ellis & Ev. Proc. Acad. Nat. Sci. Phil., 431, 1895.

(*Dothidella Castanopsidis*) Dearn. Mycologia **16**: 155, 1924.

Type collection from Sisson, Cal., on *Castanopsis chrysophylla* (Hook.) DC. Perithecial stage described from collections in Oregon on the same host, by J. S. Boyce. Collections during the past few years have shown it to be rather common throughout the California Sierras on *Castanopsis sempervirens* Dudley. Fresno Co., 1921, Bonar; Mariposa Co., 1922, Kawagoe; Tuolumne Co., 1923, Mason; Eldorado Co., 1924, Parks and Fields. These collections have included both the pycnidial and the perithecial stages of the fungus.

Single spore cultures of the fungus were started from pycnidiospores and from ascospores and grown in parallel series. The cultures from the two sources were identical in behavior in culture, in so far as could be observed. Pycnidia were formed, rather sparingly, on a number of different kinds of substrata, in both of the series of cultures. The spores from these were like those from the pycnidia on the host tissue and show agreement between the two series, so that there can be no doubt that the two described forms from this host plant are stages of the same plant.

Continued cultivation, from numerous isolations, and on a wide variety of different types of media, failed to yield any sort of reproductive body other than the pycnidia.

Leucostoma Sequoiae sp. nov.

Stromata scattered, erumpent, 2–3 mm. in diameter. Imbedded in the bark with the ostiola showing as black points. Limits of the stromata formed by a distinct black line, while the inner tissue in which the perithecia are imbedded is cinereous and fibrous in texture. Perithecia 8–12 in a stroma, in a single plane, ¼–½ mm. in diameter, with a long, slender neck which penetrates a surface disk of somewhat firmer grayish material. Asci numerous, cylindric-clavate, 45 × 7 microns. Ascospores allantoid, hyaline, not strongly curved, 8–11 × 2–3 microns.

Imperfect stage *Cytospora*, scarce on the type material. Stromata similar to those of the perithecia except that they are composed of harder, more carbonized material, as is typical for the pycnidial stage of this genus. Pycnidial stromata plurilocular, or imperfectly so, conidiophores filiform, branched, interspersed with numerous sterile hyphae, which protrude into the cavity of the pycnidium. Spores allantoid, hyaline, 4–6 × 1.5–2 microns. Spores may be extruded in yellowish masses when the twigs are moistened.

Single ascospore isolations were made of this material and it was grown in pure culture, on various types of media, for a period covering three years. Pycnidial stromata have been found in abundance in cultures but no perithecia have been formed.

On dead twigs of *Sequoia sempervirens*.

Collected Mill Valley, California, May 20, 1923.

MELANOMMA SEMINIS (Cooke & Hark.) Sacc.

Collected, Berkeley, Cal., on dead stems of *Baccharis pilularis* DC. and of *Urtica gracilis* Ait. var. *holosericea* Jepson.

The collection on *Urtica* adds a new host for this fungus.

Single ascospore isolations were made, and the fungus grows readily in artificial media of various kinds. After 7–10 days pycnidia are formed in the cultures and these, when mature, are found to belong to the form genus *Phoma*. Pycnidia globular to pyriform, papillate, 100–150 microns in diameter; spores abundant, ellipsoid, hyaline, 4–6 × 2–2.5 microns.

PHOMA THERMOPSIDICOLA P. Henn.

On leaves and stems of *Thermopsis macrophylla* H. & A., Mt. Tamalpais, Calif.

Found causing large blackened areas on the leaves in late summer. The pycnidia are scattered over the infected areas and ap-

pear on both surfaces of the leaves. About the time that the leaves are shed the pycnidia appear in abundance on the dead and dying stalks and are to be found in abundance during the winter months. This appears as a new host and regional record for this fungus which was originally described from material from the Berlin Botanical Garden on *Thermopsis fabacea*, which is native to Kamtchatka.

Phyllosticta sparsa sp. nov.

Spots circular to subcircular, brown, becoming bleached in age, surrounded by a slightly elevated brownish-black line, tissue outside this line reddish brown. Spots equally distinct on both sides of the leaf, up 3–5 mm. in diameter. Pycnidia scattered, few, on the upper surface only, sub-epidermal, erumpent, globose, reaching a diameter of 150 microns, spores globular, contents conspicuously granular, 9–12 microns. Conidiophores simple, short, up to the diameter of the spore in length.

On leaves of *Vaccinium ovatum* Pursh., Mt. Tamalpais, Marin Co., Cal., Oct., 1925.

PHYLLOSTICTA INNUMERA Cooke & Hark.

Seaver, Phyllostictales, N. Am. Fl. **6**: 71, lists this species as doubtful, as no host was given in the original note describing it in Greville. Cooke and Harkness, in their Fungi of the Pacific Coast, Bull. Cal. Acad. Sci. **1**: pt. 1, p. 14, 1884, list this fungus as parasitic on the living leaves of *Fraxinus oregana*, from Mt. Tamalpais, in California, and the type specimen, so labeled, is deposited in the Cal. Acad. of Science.

This plant agrees in every way with the description given for *Phyllosticta viridis* Ellis & Kellerm., and the name of Cooke and Hark. should supercede the later one. I have found this fungus common on the leaves and fruits of *Fraxinus oregana* in the coastal region north of the Golden Gate, and it sometimes causes severe defoliation in late summer.

Harkness listed (Bull. Cal. Acad. Sci. **1**: 160, 1885) *Phoma samararum* Desm. on the fruits of *Fraxinus oregana*. I have collected in the locality listed by Harkness and found the fruits of this tree commonly bearing the fruiting bodies of a fungus which are in every way identical with those of *Phyllosticta innumera*. This fruit infection is always associated with the leaf infection, so

that there is no doubt that the two forms are the same plant species. The fungus found on the fruits here is quite different from that described as *Phoma samararum* Desm. from Europe.

Phyllosticta Lupini sp. nov.

Spots irregular in size and shape, sometimes covering almost the entire leaflet. Upper surface little changed at first, becoming yellowish to brown, while the lower surface is black, due to the closely crowded pycnidia which are set in the leaf tissue. Effect is a slow killing of the tissue and the infected leaflet rolls inward, and in many cases a large percentage of the leaves of the plant is killed.

Pycnidia hypophyllous, globular, sub-epidermal, pushing up the epidermis so as to appear almost superficial at maturity, and very closely crowded together. Ostiole poroid. Pycnidia 100–150 microns in diameter. Conidia elliptical, hyaline, 4–6 × 2–3 microns.

On leaves of *Lupinus succulentus* Dougl., Tiberon, Calif., March, 1923. H. E. Parks. On *Lupinus micranthus* Dougl., Humboldt Co., Calif., by Parks, 1923.

Common in San Francisco Bay region, March to July.

STUDIES IN THE GENUS HARKNESSIA

Cooke, Grevillea **9**: 81, 1881; **13**: 111, 1884.

Winter, Hedwigia **22**: 19, 1883.

von Höhnel, Sitzb. Akad. Wiss., Abt. 1, **118**: 1537, 1909.

The type species of this genus, *Harknessia Eucalypti* Cooke & Hark., on *Eucalyptus globulus*, was described from material collected in California. Cooke listed the genus as one of the Melanconiales. Winter placed it in the Sphaeropsidales and has been followed by numerous writers. Von Höhnel made a study of material from European herbaria and came to the conclusion that it belonged in the Melanconiales. He listed all the numerous described forms from various parts of the world under two species—those occurring on *Eucalyptus* from various parts of the world as *Harknessia uromycoides* (Speg.) Cooke, and that on *Arctostaphylos* as *Harknessia Arctostaphyli* Cooke & Hark.

Since these two species were first collected within a few miles of each other and there seemed such very slight differences between them, I undertook a careful study of the two forms. I

have collected both these species in their respective type localities and have compared my material with the original Harkness collections in the Cal. Acad. Sci. Herbarium.

HARKNESSIA UROMYCOIDES (Speg.) Cooke.

Fruit body globoid, black, about 0.5 mm. in diameter. Sections through young ones show the upper layer to be a thin black membrane, which breaks away rather tardily, leaving a mat-like acervulus which appears as superficial, although it originated under the epidermis or periderm. Conidiophores, which remain attached to the spores when they are set free, 100–150 microns in length by 4 microns in diameter. Spores jet black when mature, with a hyaline papilla at the distal end, 24–32 × 12 microns.

Found on the leaves, twigs, and fruits of *Eucalyptus* species from California in U. S., Spain, Argentina, and Tasmania.

HARKNESSIA ARCTOSTAPHYLI Cooke & Hark.

Fruiting bodies similar to those for the above described species. Conidiophores 20–40 microns in length, with a much thickened base. Spores jet black, 18–24 × 11–13 microns, and lacking any papilla at the end.

Found on dead leaves of species of *Arctostaphylos*, Mt. Tamalpais and Berkeley, also on fallen leaves of *Arbutus Menziesii* Pursh. on the slopes of Mt. Tamalpais.

These two species have been grown in pure cultures from single spore isolations in parallel series and the morphological distinctions named above remain constant throughout the series. Fruit bodies are formed on a wide variety of media but most abundantly on those rich in sugars. *Harknessia Arctostaphyli* grows very sparingly and fruits rarely when grown on sterilized leaves or twigs of *Eucalyptus*, while *Harknessia uromycoides* fruits very abundantly on the same material. The latter species is more cosmopolitan as to its food requirements and has been widely distributed over the world with the distribution of the host species. *Harknessia Arctostaphyli* is more specific as to its food requirements and is known only from the type locality on the dead leaves of *Arctostaphylos* and *Arbutus*.

The development of the elongated conidiophores of *H. uromycoides* has been followed. The conidiophore is about the same length as the spore at the time that the spore attains approximately

its full size, although it is at that time sub-hyaline instead of black. As the spore wall becomes darker there is a very rapid elongation of the conidiophore until it reaches the lengths given for it. In a young fruit body the conidiophores will be found to be of lengths varying 25–150 microns, all bearing spores that are approximately the same size, while in the older fruit body, from which the outer wall will be broken away, the conidiophores will be found to be uniformly of the greater length, and they break away at the base, remaining attached to the spores.

Disaeta gen. nov.

Melanconiaceae-phaeophragmiae.

Acervuli intra-epidermal or subcuticular, erumpent, discoid, black; conidia elongate, fusoid, colored, with the end cells hyaline; bearing one hyaline bristle at each end of the conidium.

Like *Hyaloceros* Dur. (*Monochaeta* Sacc.) except that there is a bristle at each end of the conidium.

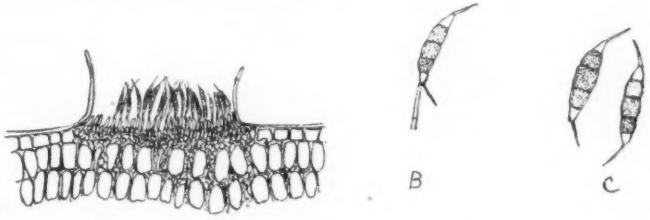

FIG. 2. *Disaeta Arbuti*. A. Section through an acervulus; B. Conidiophore with conidium attached; C. Typical conidia

Differs from *Pestalozzia* Sacc. in having the central cells colored, which places it in the Phaeophragmiae.

Type species, **Disaeta Arbuti.**

Spots irregular in outline, often becoming several centimeters in diameter and involving the major portion of the leaf, dark brown with a purplish black border, which is more evident above. Spots tend to break up and fall out in angular pieces. Acervuli epiphyllous, scattered, often concentrically arranged, .25–.5 mm. in diameter or becoming confluent; intra-epidermal, erumpent by the breaking of the cuticle. Conidia abundant, fusoid, slightly curved, typically 5-celled, the end cells hyaline, the central ones sub-opaque. Each of the terminal cells set with one bristle-like hair, averaging 7 microns in length. Conidia 18–26 × 4.5–7

microns; conidiophores simple, one-half the length of the conidia. See figure 2.

Parasitic on the leaves of *Arbutus Menziesii* Pursh., Mt. Tamalpais, Marin Co., Cal., and Oakland, Cal., Jan., 1923.

Single spore isolations were made and cultural reactions on a variety of types of food material determined. Growth is very slow on synthetic media containing various sugars as the course of carbohydrate food, and likewise on starchy food, as afforded by cornmeal agar. Growth is very vigorous on oatmeal agar and on sterilized green beans, and abundant spore formation occurred on these latter media after 1–2 months.

PESTALOZZIA CASTAGNEI Desm.

On living leaves of *Lithocarpus densiflorus* (H. & A.) Rehd. (*Pasania*) (*Quercus*), Muir Woods, Calif., Jan. 13, 1923.

Not heretofore reported on this host or from this region. Found commonly in the outer Coast Range in central California, where the host plant is found. Acervuli abundant on the upper surface of the leaves as black specks .5–1 mm. in diameter. Infection usually starts at the tip of the leaf and gradually spreads to kill the larger portion of the leaf.

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LOPHODERMIUM ABIETIS ON PSEUDOTSUGA TAXIFOLIA

J. S. Boyce

For the past 50 years species of the genera *Hypoderma*, *Hypodermella*, and *Lophodermium* and the damage they cause to young conifers have figured prominently in European literature, while within the past few years there has been an increasing interest among mycologists and forest pathologists in North America in the same fungi. New species have been described, new hosts recorded, and knowledge of distribution extended. Not only do these fungi comprise an attractive group for study but they are becoming increasingly important from an economic standpoint as the United States and Canada more and more turn their attention to timber growing. Commonly endemic on young conifers, they often become epidemic and considerable loss results.

Owing to the extremely important position that Douglas fir (*Pseudotsuga taxifolia* (Lam.) Br.) occupies and will continue to occupy in the forests of the Pacific Northwest, any parasite attacking this species demands attention. *Lophodermium Abietis* Rostrup, which attacks and kills the needles of young firs and spruces, has been reported on Douglas fir in Denmark by Rostrup (**3**, p. 527). This is the only record of this fungus on Douglas fir known to the writer, later records always referring to the observation by Rostrup. The writer has never found the fungus on Douglas fir. Throughout the Western United States *L. Abietis* is not uncommon on Pacific silver fir (*Abies amabilis* (Loud.) Forbes), white fir (*A. concolor* Lindl. & Gord.), lowland white fir (*A. grandis* Lindl.), alpine fir (*A. lasiocarpa* (Hook.) Nutt.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) where the species are mixed with Douglas fir, but the latter is not infected. In Great Britain in 1925 the fungus was found on fallen needles of Sitka spruce and Norway spruce (*Picea excelsa* Link.), but young Douglas firs in the immediate vicinity were not affected. Again, on the Island of Bronholm in Denmark no trace of the fungus was

seen in the Douglas fir plantations in the State Forest of Almindingen when they were visited in September, 1925.

The only American record the writer has been able to trace, transmitted through the courtesy of John Dearness, was a collection made by V. Simmons at Coldspring, Albany County, Wyoming, June 23, 1917. The host was labelled *Pseudotsuga taxifolia*, but a critical study of the needles proved it to be alpine fir.

Lind (**2**, p. 147) has reported the fungus on Douglas fir in Denmark, but an examination of the collection in Rostrup's herbarium at Copenhagen in September, 1925, on which this report was based showed it to be a mixture of Douglas fir and spruce needles, with the apothecia of the fungus occurring on the spruce needles only. The needles appeared to be those of Norway spruce. No other collection of *L. Abietis* on Douglas fir was found in Rostrup's herbarium. Recently (**1**, p. 486) it has been pointed out that attacks of consequence on Douglas fir have never been observed in Denmark and it is recommended that this species, because of its great resistance to the fungus, be given preference over Norway spruce for planting in the coast region.

It may be concluded that it is highly doubtful if *Lophodermium Abietis* attacks Douglas fir and past records have been based on an error in host determination.

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NOTES AND BRIEF ARTICLES

The University of Michigan Herbarium has received from Dr. Howard A. Kelly of Baltimore, as a gift, his magnificent Mycological library and his collections of photographs and paintings of mycological subjects, as well as a set of higher fungi and lichens along with a fine exhibition group of the higher fungi. It was accepted by the University on April 24. It is to be called the "L. C. C. Krieger Mycological Library and Collection" in commemoration of this artist-mycologist. About 350 paintings of mushrooms by Krieger are included. The library has an index of about 10,000 numbers. Along with the Phanerogamic and Cryptogamic Herbarium now at the University, and under the Directorship of C. H. Kauffman, the Kelly gift is installed in the New Museum Building just completed at Ann Arbor. It is expected to be available soon to the scientific public.

C. H. KAUFFMAN.

THE C. G. LLOYD MYCOLOGICAL COLLECTION

During Mr. Lloyd's last visit to Washington he expressed a desire to have his Herbarium brought here, and tentative plans were made. Later, on account of ill health, he decided that he would be unable to carry out the plan. Since Mr. Lloyd's death, a coöperative arrangement has been made between the Smithsonian Institution and the Department of Agriculture to take charge of the collection, and the trustees of Mr. Lloyd's estate have transferred the collection to Washington, where it will be installed in fireproof cases in the Bureau of Plant Industry, as a separate unit in connection with the present Mycological Collections.

This collection represents the life work of Mr. Lloyd, and contains according to his estimate about 100,000 specimens, consisting chiefly of the larger Hymenomycetes and the larger Ascomycetes. Many type specimens are included not only of species described by Mr. Lloyd, but also those of other mycologists; also nearly 10,000 negatives illustrating fungi, hundreds of

photographic prints and half-tones of all illustrations published by Mr. Lloyd, and his voluminous correspondence with most of the mycologists of the world. All of the material has been safely received in Washington, and will be catalogued, arranged and made available for study as soon as practicable.

The collection is particularly rich in material from all parts of the Tropics and little-explored regions of South America, Africa, Australia and other countries. C. L. SHEAR.